# Microbial Source Tracking in Vermont Using Ribotyping of *Escherichia coli* Isolates

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## EXECUTIVE SUMMARY

Water and feces samples from a variety of species were collected from two watershed areas adjacent to Lake Champlain in Vermont during August, 2000. *Escherichia coli* strains were isolated from these samples and sent to the University of New Hampshire Jackson Estuarine Laboratory's new ribotyping facility. The DNA of all culturable strains sent from Vermont were processed for ribotype profile analysis to identify source species for isolates from water samples, using the isolates from feces samples as references. The data were analyzed to provide information with a range of degrees of certainty for the relatedness between known source species and water sample profiles. As expected, the more strict requirements for matching of profiles, the fewer matches. The results provide a guide for what species are significant sources at the 13 different sample sites in the two watershed areas. Using only the Vermont source profiles (library) and a conservative, defendable set of source species profiles and analysis guidelines, the group of wildlife species, including seagulls, raccoons and mallards, was the most common type of source species, even though the avian species only occurred in the Colchester area. Septage was only identified in the Winooski area. Cats and cows were also rarely identified source species in both areas. Overall, seagulls were the most commonly identified source species. However, use of a larger set of NH source species profiles combined with the VT database resulted in more identification of water sample profiles and a different mix of identified source species. Humans/septage was the most common individual source species and was found in both study areas. Grouping of individual source species into types showed wildlife to be the largest category of identified source species, while avian species diminished in prominence. The NH source species database also included species not included in the VT database that proved to be important, especially deer. These results emphasize the importance of a large database with a wide variety of source species. This small study should provide useful information for the management of fecal contamination in the two study areas. This study is also an important and useful early step for using ribotyping for identifying fecal contamination sources in northern New England watersheds.

## INTRODUCTION

One of the most common issues facing environmental managers concerned with surface water quality is fecal-borne microbial contamination and the threat of diseases to humans who come in contact with water or shellfish from contaminated areas. For purposes of monitoring the sanitary quality of surface waters, fecal coliforms, enterococci and *Escherichia coli* have served relatively well as indicators of water quality for classifying waters to protect public health. However, as many of the obvious sources of contamination, such as untreated sewage from poorly run wastewater treatment facilities, have been eliminated or reduced in significance, the residual contamination that limits uses of surface waters is often of unknown origin. Efforts to reduce contamination have often revolved around making a best guess of what potential sources may be significant, conducting extensive sampling programs, eliminating sources and then resampling surface waters to see if improvements in water quality have occurred. This process is expensive and oftentimes less fruitful than desired.

Recent adoption of biotechnological techniques for application to water quality issues has spawned a number of approaches to address identification of sources of fecal-borne contamination. These new approaches, often called "microbial source tracking" (MST), have been used for over 10 years in a number of areas in the U.S. with success. Use of ribotyping of *E. coli* isolates cultured from target surface waters is one approach that can provide detailed information on sources of fecal contamination and has advantages over other MST methods.

Various studies have reported on the use of ribotyping for tracking sources of fecal-borne microbial contaminants. The approach involves identifying microorganisms in the environment as being from different sources by comparing patterns of DNA fragments isolated, digested by restriction enzymes and electrophoresed. The method requires analysis of DNA fragments from the unknown surface waters compared to isolates from known sources, including all human and animal sources suspected of being in the watershed. Samadpour (1995) used ribotyping of *E. coli* from either livestock on hobby farms or on-site septic systems in Washington State. Numerous ribotyping studies have been conducted in freshwater watersheds (Carson et al., 2001; Barsotti et al., 2000; Hartel et al., 1999; Tippets, 1999; Berghoff, 1998), while others have been conducted in estuarine waters (Parveen et al., 1999; Samadpour, 1995; Simmons et al., 1995). The Barsotti et al. (2000) study was located in Shelburne Bay, Lake Champlain, VT, just south of the study area for this report.

## PROJECT OBJECTIVES

The goal of this project was to use ribotyping to identify the most significant sources of fecal contamination in the two study areas. The results should be useful as a basis for effective resource allocation and management activities to eliminate those sources and improve water quality. The specific project objectives were as follows:

1) Identify sources of bacteria to Malletts Bay and the Lower Winooski River;
2) Contribute to development of a regional DNA source library for *E. coli*;
3) Strengthen/refine capacity for ribotyping in New England by coordinating protocols with other regions of the U.S. where such protocols are available.

## MATERIALS AND METHODS

### Site Characteristics and Sample Timing

The study was conducted in the Malletts Bay and Lower Winooski River watersheds next to Lake Champlain in northern Vermont (Figure 1). Figure 2 shows the water sampling stations in the two main areas. The sample stations were named either as "C" (Colchester) or as "W" (Winooski) sites. Not all of the sites shown were included in the ribotyping portion of the overall project. The names and descriptions of sites included in the ribotyping study are listed in Table 1, based on information taken from the project QAPP and a previous project report (ABS, 2001).

The Colchester area sites are all located in close proximity to the Lake Champlain shoreline, in or near drainages to Malletts Bay and along the shoreline of the main lake (Figure 2). The Winooski area sites are all in or near the downtown portion of the city of Winooski near the Lower Winooski River. The Colchester sampling stations include stream, brook, river and lakeside sites (Table1). The Winooski sampling stations include a brook site, a river site (the Winooski River) and 3 storm drains outlets.

The samples collected for ribotyping analysis were all collected during a seven-day period in late August, 2000 (Table 2). The three sampling dates (8/22, 8/23 and 8/29) span a modest storm event that occurred on 8/23. At a number of sites (C2, C5, C13 and W2) samples were obtained on all three dates -- before, during and well after the storm event. In all cases except one (C9 -a site in Outer Malletts Bay minimally influenced by stormwater) at least one wet weather and one dry weather sample was obtained at each site. In addition, a number of the Winooski sites (W1, W2, W6 and W8) were sampled at two different times during the storm event (first flush = "FF" and mid-storm) as many of these are urban stormdrain sites that experienced significant flow increases during the storm. Flow levels at some of the more rural Colchester sites increased less during the storm, and these sites were sampled only once on 8/23/00 near the end of the event.

### Isolate Transfer from Vermont Study Sites

*Escherichia coli* strains from the sampling sites were collected for ribotyping analysis on three dates: August 22, 23 & 29, 2000. Strains were isolated from enumeration agar plates and subject to biochemical tests to confirm their identification as *E. coli* isolates. The isolates were frozen in cryovials containing saline/DMSO/glycerol preservation media and the vials were packed in boxes on dry ice. The isolates were mailed to UNH/JEL in early September, 2000. *E. coli* isolates were also collected from feces samples, collected during late summer and early fall, and mailed to UNH in November, 2000. All of this work was conducted by other participating investigators (ABS, 2001) prior to the receipt of isolates at UNH/JEL.

Upon receipt of isolates from Vermont at UNH/JEL, the boxes of vials were unpacked and examined for problems during shipping; no problems were noted. The vials were immediately stored at -80°C until processing for ribotype analysis.

Sample Processing

The procedures used for ribotyping *E. coli* isolates for this study are based to a large extent on those of Parveen et al. (1998) and more detailed protocols developed and kindly provided by Dr. Peter Hartel of the University of Georgia. The *E. coli* isolates in the cryovials from Vermont were thawed and re-cultured onto trypticase soya agar (TSA). Some of the isolates could not be re-cultured. Cultures on TSA were incubated overnight at room temperature (~20°C). Some of the resulting culture was transferred to duplicate cryovials containing fresh glycerol/DMSO cryo-protectant media for long-term storage at -80°C.

E. *coli* isolate cultures were used for DNA extraction. Extraction was performed using Puregene (Gentra) kits and the manufacturer's instructions. Briefly, 5 ml of overnight cultures was centrifuged at 10,000 rpm for 5 minutes to concentrate the cells from the liquid medium. 300 $\mu$l of lysis solution was added to the pelleted cells, mixed and incubated for 5 minutes at 80°C. 1.5 $\mu$l of Rnase solution was added then incubated at 37°C for 15-60 minutes. A protein precipitation solution was added, then the tube contents were mixed and centrifuged at 13,000 x g. The supernatant was transferred into a clean tube. Isopropanol and ethanol were added to remove DNA, and a hydration solution was added to re-hydrate the DNA at 65°C for 1 h, then stored at 4°C.

The resulting DNA for each isolate was quantified by fluorometer (Turner TD700) using Hoesct's dye and calf thymus DNA at 100 $\mu$g/ml as a standard. DNA concentrations were recorded on the vials, in a lab notebook and in a computer database.

Restriction of the DNA was conducted using EcoRI (Sigma) and the manufacturer's instructions. Briefly, 2 $\mu$g of isolate DNA, 2 $\mu$l of the appropriate 1x buffer and 0.5 $\mu$l of EcoRI restriction enzyme were added to a 0.5 ml tube. Autoclaved diethylpyrocarbonate (DEPC; Sigma) water (0.1%) was added (~16 $\mu$l) to bring the total volume in the tube to 20 $\mu$l. The mixture was incubated overnight at 37°C. The next morning, 0.2 $\mu$l of EDTA was added to stop the reaction.

Gel Electrophoresis, Probe Hybridization and Detection

Restriction-digested DNA was separated by sub-marine gel electrophoresis (EC App. Corp.) in Tris-acetate-EDTA (TAE) buffer. Volumes (12 $\mu$l) of positive and negative control, isolate and standard samples were loaded into 0.8% (Nu-Seive 3:1) agarose gels. Denaturation, neutralization and Southern blotting were performed using a Vacugene XL (Amersham). When the transfer was complete the membrane was washed, placed on blotting paper then crosslinked (Spectrolinker XL1000).

A probe was made as follows. In a 2 ml tube, 20 $\mu$l of 16S 23S rRNA (Sigma), 2 $\mu$l of DEPC water, 2 $\mu$l of reverse transcriptase (Sigma), 8 $\mu$l of 5x buffer, 4 $\mu$l of dNTP (Roche) and 4 $\mu$l of hexanucleotide mix (Roche) were mixed together. The solution was incubated overnight at 42 °C.

Prehybridization was performed in an Isotemp (Fisher) hybridization oven at 42°C for 2 h, using 30 ml hybridization solution per membrane. The probe was denatured by boiling for 10 minutes and rapid cooling in an ethanol-ice bath. The probe was added to 30 ml pre-warmed hybridization solution and incubated for 30 minutes at 68°C. The original hybridization solution

was poured off the membranes and the probe solution was added and incubated overnight at 42°C.

For probe detection, the membranes were then subject to a series of stringency washes. Blocking was done at room temperature for 60 minutes and the solution was poured off. Freshly prepared anti-DIG solution was added, incubated for 30 minutes at room temperature and poured off. Tween buffer was added and incubated for 15 minutes at room temperature. Detection buffer (Roche) was added and incubated for 2 minutes. The membranes were then placed into an acetate sheet and 20 drops of CDP-Star (Roche) was added and incubated at room temperature for 7 minutes.

Image Digitization, Optimization and Band Identification

Processed membranes were placed into the darkroom of an Epi Chem (UVP) chemiluminescence imager and the image was digitized with a 12-bit CCD camera. Each image was converted to 16-bit data, inverted and the display range set with LabWorks software (UVP).

The images were transferred into GelComparII (Applied-Maths) analytical software and the lanes for each gel were visually demarcated. The bands in lanes containing the standard were labeled and entered into the memory for optimization of gel pattern images. Densiometry data were processed for band identification. A representation of data output is illustrated in Figure 3.

New Hampshire Source Library

Fecal samples from source species in watersheds in coastal New Hampshire were processed for isolation of *E. coli* strains. The isolates were then subject to the same, previously described procedures used for all isolates. A new database containing all NH and VT source species profiles with > 2 bands was used to analyze the profiles from water sample isolates and results were compared to results using only the VT database.

Statistical Analysis

Individual 'unknown' isolate data were selected from the computer database for identification of source species. The entire Vermont library of isolate profiles for known source species was used for comparison with each unknown isolate, excluding in successive analyses those profiles with only one band, then those with <4 bands. Similarity indices between the unknown isolates and the known source isolates were determined by using Dice's coincidence index, using 2% for tolerance and optimization settings. For the combined NH & VT database, more stringent tolerances (0.5-1.5%) were used to enable differentiation between profiles that initially yielded matches with the same % similarity coefficients using 2% tolerance. The source species profile with the best similarity coefficient at a more stringent tolerance was accepted as the source species.

Cluster analysis was used to determine the relationships among isolates from the same sources and the same sites, as well as banding patterns that were identical for different isolates.

## RESULTS

Isolate Recovery

Of the 176 isolates from water samples received at UNH/JEL, 172 were successfully re-cultured. There were also 308 isolates from known source species sent to UNH/JEL. Of these, 261 were successfully re-cultured, and 47 were not recovered. There appeared to be one box (ABS 9) of isolates in particular that had almost all (42) of the isolates that were not recovered. Unfortunately, none of the beaver isolates were successfully re-cultured. There were isolates recovered to represent all the 9 other source species.

Ribotyping Success

Ribotype banding profiles were determined for all culturable isolates. Initially, acceptable banding profiles ranged from 2 to 12 bands. Isolates for which there was only one band were re-analyzed. All isolates with only one band after reanalysis were removed from the database. In fact, many of them matched other isolates because the position of the single band was identical, and would have been discarded for not being unique to any one source species.

Isolates from known source species

The total number of re-cultured isolates from known source species that were processed for ribotyping was 261 (Table 3). Of these, ribotyping (>1 band) was successful for 209 isolates, or 80% of the total. Success varied for the different source species isolates from 55% for horses (11 of 20) to 94% for raccoons (33 of 35). The number of isolate ribotypes available per species ranged from 8 for pigeons to 41 for cows.

Isolates from water samples: 'Unknowns'

The results of the similarity analyses for all sites on each date are summarized in Table 4. The actual similarity coefficient is given on the right side of the table along with the number of bands for both the unknown sample and the matching known source profile. On the left of the table, the identified source species is given for each of four similarity percent ranges.

There were 172 isolates from water samples from the 13 different sample sites (Tables 5a-c). Ribotyping was successful for 132 isolates (excluding profiles with 1 band), or ~77% of the total isolate cultures. There was at least one ribotyped isolate for every site/time, with a range of 1 to 8 (site C7 on 8/29/00) isolates, not including the 9 isolates for sites W1 and W2 on 8/23/00 when they were sampled twice on the same day. Tables 5 a-c show expected (Johnson et al., 2002) decreasing numbers of isolates with identified source species as the acceptable percent similarity coefficients are increased.

Source Species Identification

Based on the variability observed for the standard *E. coli* isolate ribotyping results, as well as the relatively low number of known source species profiles in the database, a similarity of 80% or greater between unknown isolate patterns and those of known isolates was used to identify source species. This translates to differences in approximately 1-2 bands for most profiles. Some of the results included highly similar patterns from more than one source species. These results were considered unsuccessful in that no single source species could be attributed to these profiles.

The results are presented in summary tables of analyses using Dice's coincidence index and 80, 85 and 90% similarity coefficients for acceptable matches to source species. The number of identified source species decreased from 44 to 26 and 18 as the acceptable similarity coefficient decreased from ≥80% to >85% to >90%, respectively. Table 5a summarizes the number of isolates for which similarity coefficients for unknowns were ≥80%. The total number of isolates with identified source species was 44 (~33% of the 132 total ribotyped isolates), while 88 (~67%) isolates were classified as having no identifiable source species, i.e., 'unidentified'. The number of isolates with ribotypes and identified source species split relatively evenly between the two areas. There were 70 isolates ribotyped from the Winooski area and 62 in the Colchester area, while the same (22) number of identified isolates occurred in each area. Thus, the percentage of identified isolates for each watershed was 31 and 35% for the Winooski and Colchester areas, respectively.

Table 5b summarizes the number of isolates for which similarity coefficients for unknowns were >85%. The total number of isolates with identified source species was 26 (~20% of the 132 total ribotyped isolates), while 106 (~80%) isolates were classified as 'unidentified'. There were 12 identified isolates from the Winooski area and 14 in the Colchester area. Thus, the percentage of identified isolates for each watershed was 17 and 23% for the Winooski and Colchester areas, respectively.

Table 5c summarizes the number of isolates for which similarity coefficients for unknowns were >90%. The total number of isolates with identified source species was 18 (~14% of the 132 total ribotyped isolates), while 114 (~86%) isolates were classified as 'unidentified'. There were 8 identified isolates from the Winooski area and 10 in the Colchester area. Thus, the percentage of identified isolates for each watershed was 11 and 16% for the Winooski and Colchester areas, respectively.

The identified source species for 'unknown' water isolates are summarized in Tables 6a-c. The results show the percentages of both unidentified and the identified source species for each site. At 80% similarity (Table 5a), there was at least one identified isolate for each of the nine source species used. The source species with the highest rate of occurrences were seagulls and raccoons, with cats, cows, mallards and septage at an intermediate level of occurrence, while dogs, horses and pigeons were rarely identified as source species. In Tables 6b & c (85 & 90% similarity, respectively), all source species except for dogs are still identified sources for at least one sample, with decreases in occurrences for most of the other source species.

Tables 7a-c show the number of isolates identified to each source species in the two watershed areas. In the Colchester area, seagulls, raccoons, cows and cats occurred in more than one sample (80% similarity; Table 7a), with no horses or pigeons. The Winooski area differed in that septage also occurred in more than one sample, isolates matched once for both horses and pigeons, and no matches occurred for dogs. There were no drastic differences in the numbers of

isolates matched to any given source species between the two areas. Seagulls were the only source species where there was a difference of more than one isolate (Colchester>Winooski).

At 85% (Table 7b) similarity, the numbers of isolates identified to each source species in the two watershed areas generally decreased. There were two more mallard isolates in the Winooski compared to the Colchester area, otherwise, incidences were nearly the same for the two areas. As previously mentioned, dogs were no longer included as an identified source species. At 90% (Table 7c), the number of isolates identified as cats, raccoons, seagulls and septage decreased even more. The biggest drop in occurrence was from 7 to 2 isolates for sea gulls in both areas. Tables 8a-c summarize the occurrences of the identified source species for isolates by site and sample time.

Temporal Trends and Storm Effects

The database is limited in terms of number of sample dates. As previously discussed, most sites were sampled on only two of the 3 days, only the Winooski sites were sampled twice on 8/23/00, and one site was sampled on only one date. These factors make it difficult to analyze the data for temporal trends, other than to see if any source species are found on more than one sample date or time.

Tables 8a-c summarize the occurrence of source species identification for water sample isolates at each site and sample time. The far right column in each table is a summary of the identified source species found at each site that reoccurred in different samples. In the Colchester area, raccoons occurred on 2 dates at site C4, while seagulls and mallards reoccurred at site C5. In the Winooski area, seagulls reoccur at site W1, raccoons at site W2, septage at site W6 and cats at site W8. With the decrease in occurrence of identified source species with increasing similarity coefficients, the reoccurrence of source species at sites also decreased. At 85% similarity, only raccoons at site C4 and seagulls at site W1 reoccurred (Table 8b), while at 90% similarity (Table 8c) only seagulls at site W1 reoccurred.

There was only one instance of isolates from the same source at a site during the two sample times on 8/23/00 at the Winooski sites; septage was identified as a source at W1 during both the mid-storm and the first flush (FF) sample time (Table 8a). Otherwise, the identified source species were not consistent for the two sample times on 8/23/00 for any one site. The paucity of data limits any further trend analysis.

The small database allows for some initial assessment of storm event effects on sources compared to dry weather conditions. Taking another look at Tables 8 a-c reveals the incidence of source species under either dry weather (8/22 or 8/29) or under rainstorm (8/23) conditions. The question that is of interest is, are source species different under the two types of conditions? In general, there are no sites where the identified source species were exactly the same under dry and wet conditions. In some cases, there was no similarity. The reoccurrence of source species has been summarized above, but can also be looked at relative to wet-dry conditions. For the Colchester area at 80% similarity and all profiles >1 band, there were 3 sites where source species were identified on dry and wet sample dates: C4, C5 and C7. There were 2 sites where one (raccoon; C4) or two (seagull & mallard; C5) source species occurred both under wet and dry conditions. Again, there were other source species that did not reoccur at both sites. For analyses using 85 & 90% similarity, the database diminishes and fewer conclusions can be made. Overall,

there were more identified source species for dry conditions compared to wet conditions, suggesting a wider diversity of sources. Septage, cows and dogs did not occur under wet but did occur under dry conditions at the 3 sites with source species for both conditions. For profiles only with >3 bands, there were 2 sites where source species were identified under both wet and dry conditions, and site C5 again had a reoccurrence of seagulls under dry and wet conditions. There were slightly more source species under wet compared to dry conditions.

In the Winooski area for profiles with >1 band, 4 sites had identified source species under both dry and wet conditions. There were reoccurring source species identified at 3 sites, W1, W2 and W8, but in each case there were other source species that occurred only under either dry or wet conditions. Overall, there were more identified source species under wet compared to dry conditions, with cows only occurring under wet conditions and mallards only under dry conditions. For profiles with >3 bands, only one site had identified source species for both dry and wet conditions, and the species were different for each condition.

Similarity Analysis Between and Within Source Species Profiles

Intra-species comparisons

The ribotype profiles for the isolates of each source species were analyzed using Dice's coincidence index and cluster analysis to determine ribotype diversity and the frequency of identical patterns. Profiles with one band were excluded from the database. Source species profiles were considered to be matches if they were identical (100% matching). The results are summarized in Table 9. Of the total of 186 source species profiles, there were 12 pairs that matched. There were 3 matched pairs each for mallards and cows, while septage, cats and pigeons had no matching pairs.

The diversity of ribotypes can be represented by the clone:isolate ratio (Berghoff, 1998). This is simply the ratio of unique profiles to total isolates for each source species; higher numbers suggest more diversity. Ratios ranged from 67% for horses to 100% for cats, pigeons and septage (Table 9). The overall average ratio was 91%.

Interspecies comparisons

The ribotype profiles for the isolates from all source species were analyzed using Dice's coincidence index and cluster analysis to determine if any profile patterns are the same for isolates from different species. It is important to exclude these patterns from the database because they are not useful for identifying unique source species for water sample profiles. Profiles with one band were excluded from the database. Profiles were considered to be matches if they shared 100% matching. The results are summarized in Table 10. Of the total of 186 source species profiles, there were 17 instances where profiles matched for either 2 or 3 different source species. The number of profiles that were included in each matching incidence ranged from 2 to 5, and the number of bands ranged from 2 to 4. Gulls and mallards were involved in 4 matches, raccoons in 3, horses in 2 and cats, septage, dogs and cows in 1 match each. These results were reflected in the analysis of profiles of isolates from water samples. There were 4 examples (all at site W2 sampled mid-storm during 8/23/00) where isolates were matched (100%

similarity) to more than one source species and thus their source species could not be identified.

Similarity Analysis of Water Samples Profiles Within and Between Sites

The occurrence of matching profiles was rare for 'unknown' water sample isolates within each site. The data are not summarized in a table because, of the 4 occurrences, only one was for a profile that also matched a source species profile at >80% similarity. The other within-site matches had lower similarities to source species profiles or were similar between two different species. The only site with reoccurring profiles also matched to source species was C2. The source species was seagulls, and the two matches occurred on 8/23/00. Further analysis of the entire 'unknown' database for matching profiles between sites showed a mallard profile occurred at sites W1 and W6 on 8/23/00 during the first flush (FF) and the "mid-storm" sample times, respectively. The same mallard profile also showed up at site W11 on 8/29/00.

Similarity Analysis of Water Samples Profiles With >3 Bands

Another approach that should be considered is to exclude all profiles with less than 4 bands. This excludes questionable profiles and makes the overall analysis of host species identification more robust by using just profiles with 4 or more bands. The same analysis of the results presented previously for all profiles with >1 band is reiterated to some extent in this section. The level of similarity used for acceptable matches is 80%.

Table 11 shows the overall results for analysis of profiles with >3 bands. There are obviously fewer matches, 20, compared to the results (44 matches) summarized in Table 4 that also included matches for profiles with 2 or 3 bands. Only 3 of the 20 matches have % similarity >90%, and 11 of the 20 have similarities <85%.

There were 172 isolates from water samples from the 13 different sample sites (Tables 5a-c). Ribotyping was successful for 66 isolates (excluding profiles with <4 bands), or ~38% of the total isolate cultures (Table 12). Of the 66 acceptable profiles, 20 (30%) were identified to source species and 46 (70%) were unidentified. There were numerous instances of no ribotyped isolate for some site/time samples. There were 40 isolates ribotyped (>3 bands) from the Winooski area and 26 in the Colchester area, with 13 identified in the Winooski area and 7 in the Colchester area. Thus, the percentage of identified isolates for each watershed was 32.5% and 26.9% for the Winooski and Colchester areas, respectively.

The identified source species for 'unknown' water isolates with >3 bands are summarized in Table 13. The results show the percentages of both unidentified and the identified source species for each site. There was no identified isolate for horses, pigeons or dogs in either watershed area. Four sites, C9, C12, W1 and W11, also had no identified isolates. These sites had some of the lowest numbers of acceptable isolate profiles. The overall occurrence of identified source isolates was 30%, with occurrences of species ranging from 2% for mallards to 11% for seagulls. The occurrences of the five general categories were 3% for septage (humans), 5% for pets (cats), 5% for livestock (cows), 13% for avian species (seagulls and mallards) and 6% for wildlife (raccoons).

The source species with the highest rate of occurrences was seagulls (7), followed by raccoons (4), cats and cows (3), septage (2) and mallards (1) (Table 14). Cats, raccoons and cows occurred in both watersheds. Seagull and mallard isolates only occurred in the Colchester area, while septage isolates only occurred in the Winooski area. Finally, the exclusion of profiles with <4 bands produced fewer acceptable profiles for source species. Only 109 (52%) of the 209 host species isolates that were ribotyped had >3 bands (Table 15). The 3 species with the lowest number of profiles with >3 bands, horses (1), pigeons (3) and dogs (8), also were the species that were not identified as sources species.

Analysis of Water Samples Profiles (>2 bands) Using a Combined NH & VT Database

The profiles with >2 bands from the VT source species database were combined into a new database with profiles with > 2 bands from a NH database. The fraction of NH isolates that have been ribotyped is 48% (Table 16), although some of the isolates are still being re-analyzed. Different species had varying levels of ribotyping success. For example, only 2 of 15 NH duck isolates were ribotyped, while 21 of 25 NH geese isolates were ribotyped. However, of the 245 ribotyped isolates, a high fraction (83%) of patterns had >2 bands.

The new combined database was used to analyze profiles from water samples collected from the 2 VT watersheds. Table 16 shows the 21 different source species from which fecal samples were collected and analyzed from NH, and includes a list of the number of isolates with >2 bands from the VT source species isolates. The total number of useable ribotype patterns in the combined database is 349, with about 58% from NH and 42% from VT. Isolates for nine of the source species came only from NH, isolates from one species were only from VT, three source species had no isolates with >2 bands and eight source species had isolates from both areas.

The results of analyzing each water sample isolate with >2 bands collected on the 3 sample days are shown in Tables 17 a-c. The results show successful identification ($\geq$80% similarity) increased from 8/29 < 8/22 < 8/23. For each sample date, addition of the NH isolates into a combined source species database resulted in a greater number of water isolates having matching source species at $\geq$80% similarity. In general, use of the combined VT/NH source species database increased the number of identified sources for water samples from 27 to 50 (bottom of Table 17c). The 50 samples with identified source species represents 54% of the total 93 samples with patterns having >2 bands. In most (30 of 50) cases, the combined database best-fit ribotype pattern was from a source species that differed from the source species indicated from analysis using only VT isolates. In some of these cases the best-fit pattern was from a source species not originally included in the VT database.

The overall results are also summarized in Table 18, which illustrates successful identification of source species for samples collected at each site, watershed and date. Each site except c12 had at least one identified source. The fraction of ribotyped isolates with matching source species patterns with $\geq$80% similarity for each watershed was 53-56%.
Table 19 summarizes the results of source species identification for each watershed and site. Overall, 14 of the 16 source species in the combined database were identified as sources with only cormorant and NH-duck as exceptions. The fraction of isolates from which identification of source species was successful was $\geq$50% in 8 of the 13 sites. Raccoons were identified as

sources at 7 sites, septage and deer at 6 sites, cats at 5 sites, cows at 3 sites, geese and seagulls at 2 sites and dogs, mallards, horses, chickens, coyotes, foxes and muskrats at 1 site each (Table 19).

Inclusion of the NH source species profiles in a combined database resulted in an increase of 16 additional identified ribotypes for species not included in the original VT database (Table 20). There were 11 different source species identified at sites in the Colchester area watershed and 9 in the Winooski area watershed. However, there were differences in the presence and absences of source species in the two areas. Dogs, horses and foxes were not identified sources in the Colchester area but were present in the Winooski area, while mallards, seagulls, chickens, coyotes and muskrats were present in the Colchester area and absent in the Winooski area. Source species decreased in frequency in the following order: septage>deer>raccoons>cats> cows>seagulls>chickens & geese>dogs, horses, mallards, coyotes, foxes and muskrats. Pigeons, cormorants and ducks-NH were not identifed as sources.

Comparison of results in Table 20 between different databases shows how inclusion of the NH database changed the frequency and occurrence of identified sources species. As expected, allowing for one less band in profiles expanded the source species database and more positive identifications resulted (50 compared to 27). The prominence of cats, cows and especially seagulls diminished while that of septage/humans increased. The inclusion of nearly one third (16 of 50) of the total as new species (only included in the NH database) suggests that a wider array of species was needed for the study area.

The different species can be grouped by type of source. Table 21 shows how the results of analysis using the combined NH/VT database suggested source species in 6 different groups. The largest fraction of ribotyped isolates were 'unknowns'; 46% of all ribotypes, and 45 or 47% in the 2 study areas. The three most frequent suggested source species groups are 'wildlife' at 20%, 'septage/human' with 12% and 'livestock/chickens' with 8% of the total ribotypes. Each of these groups had similar percentages in the two study areas. 'Pets' and 'birds' had 8% and 6%, respectively, of the total ribotypes with suggested source species, but they differed between the 2 study areas. Pets were more prevalent in the Winooski area and birds were more prevalent in the Colchester area.

## DISCUSSION

The ribotyping results of this study can be used as a guide for helping to direct pollution source remediation in the two target watershed areas. However, the source species database is relatively small, water sampling was limited to 1-4 sample times that differed for the different sites, and the results for ribotyping reflected the developing nature of the process at the UNH lab. This study is the first one completed for the new ribotyping facility at UNH/JEL, and the results reflect some early process modifications and optimization. However, the nature of much of the findings and laboratory results is similar to what has been reported in other studies and by other researchers. The analyses and interpretations have benefited from recent input and communications with other ribotype researchers, in a continuing attempt to improve application capabilities for ribotyping in this region.

Discussion of Results Using Only the Vermont Source Library

The results show that successful identification of water sample profiles to host species ranged from 14% of the total isolates using 90% similarity, 20% using 85% and 33% using 80%. The range of ribotyping success of this study is similar to other studies from laboratories conducting ribotyping. For example, the percentage of isolates identified to source species was 19% in the study by Berghoff (1998). The big difference between that study and this one is that no source species samples from the study site area were taken. The source species database used, that of M. Samadpour, was much larger than the database for this study and it was made up entirely of isolates from areas other than Glen Canyon. In the Barsotti et al. (2000) study in northern Vermont, 28 of 57 (49%) isolates were identified to source species. That study was quite different from this study in that water samples were from the drinking water inlet pipe that pumps from the bottom (75 feet) of Shelburne Bay/Lake Champlain, 2480 feet from shore. The identified source species were similar to those found in this study, and included humans/sewage, cows, avian species (seagulls, ducks, geese) and a small number of deer/elk isolates. The study by Samadpour and Chechowitz (1995) reported 59-80% matching of isolates to source species in different areas of the watershed they were studying, again using a large source species database of profiles.

One of the main concerns was how to set a level of similarity to accept for matching water sample profiles to the host library profiles in order to identify source species. The report summarized analyses at 3 different similarity coefficients, ≥80, 85 and 90%. Johnson et al. (2002) also reported results of analyses using a set of similarity threshold values ranging from 80-95%. The ultimate decision on what level should be used needs to be based on a number of criteria. First, we considered the inter-gel variability by using Dice's coincidence index to analyze patterns for our *E. coli* positive control. There is also the need to realize that one mutation that could cause changes in the banding pattern for what are otherwise isolates with the same ribotype profile would give lower levels of similarity, especially for strains that have fewer bands (Coastlines, 1998). We also realize that, for studies that have <300 host isolates (such as this study), it is difficult to see results when a stringent, or higher level of accepted similarity is used (personal communications with many ribotype researchers). Finally, the tolerance and optimization settings can be used to off set the similarity coefficient used. All of these factors were considered in the process of selecting similarity threshold values for identifying source species in this study.

Despite the various limitations of the study, the results appeared to give some interesting results. For example, only three water sample isolates were identified as being from septage in the two areas, even at 80% similarity. This may be a reflection of what type of 'septage' sample was collected and where it came from. Some septic systems may contain *E. coli* from cats and dogs if owners put waste in the toilet. The septic systems sampled in this study were chosen to not have pet feces as part of their waste streams. Sewage treatment facility effluent, although not included in this study, can also contain *E. coli* isolates from pets or other. There was evidence for livestock (cows) and pets (cats) contributing to contamination in both areas, but the wildlife species, mallards, raccoons and seagulls were the most common identified type of source.

With such a small source species database, it is possible that the number of identified isolates was in part a function of the number of source species ribotype profiles in the database.

Table 2 shows that the most abundant source species were, in decreasing numbers, cow, raccoon, seagull, septage, mallard, cat, dog, horse and pigeon. This order reflects the frequency of identified source species to an extent, although not precisely. Another way to consider the results is that the abundance of source species in the database also reflects the frequency of those species for which feces was present in the watershed. From that standpoint, the identified source species for water samples would be representative of sources in the watersheds. This assumes that source species for which no feces was collected, such as rodents, are not significant. Ideally, ribotyping studies benefit from having much larger source species databases. A better variety of possible unique banding profiles for each source species would increase accuracy and the probability that sources can be identified for water samples. The number of source species banding profiles needed to overcome any bias resulting from the number of profiles in the database is not known, but the number in common usage as a guide is >300.

Somewhat different results were observed when using only profiles with >3 bands. Using the criteria of 80% similarity for profiles with >3 bands, identification success was 30%, although this is based on a much smaller number of accepted profiles (66 compared to 132) for unknown isolates. The rarest occurring source species, horses, pigeons and dogs, were no longer included as identified source species. Again, the wildlife species were the most common source species, but the avian species only occurred in the Colchester area. Septage was only identified in the Winooski. The results based on profiles with >3 bands are the most defendable, but the resulting decrease in acceptable profiles makes it more difficult to identify source species with confidence.

The occurrence of isolate profiles with only one (or even 2-3) band is not unexpected, but the need to reject their use in the analyses reduces the usefulness of the samples collected for identifying pollution sources. There are several reasons for getting one or a few bands. There may actually be a limited number of sites the enzyme can cut, the suspected *E. coli* may be another bacterial species, or there may have been other problems in the processing of the DNA. It is possible that some of the isolates used were not *E. coli* because of the probability, though low, that an isolate would be a false positive after the different biochemical analyses conducted to confirm their identification. Theoretically there could be a relatively high number of false positives for a given sample area/date if a prevalent and thus frequently isolated strain produced misleading results. To address the problem of profiles with 1-3 bands, some ribotyping labs use multiple (usually 2) restriction enzymes to digest host and sample isolate DNA. In that manner, an isolate that only has one band using EcoRI, the enzyme used in this study, may have more bands using another enzyme and thus be useful. This would have required us to do twice the work at twice the expense. In the future, depending on the ability to upgrade and speed up our analytical capabilities, we will probably also use two restriction enzymes.

Discussion of Results Using the Combined NH and VT Source Library

An ongoing project in New Hampshire has recently completed a large library of known source isolate profile data. The data from that library was used in conjunction with the library compiled for the VT samples for comparative identification of sources of fecal contamination in the Malletts Bay and the Lower Winooski River watersheds. Use of a larger, regional library would hopefully increase the successful identification of source species for water sample isolates.

This also helps to address the question, how applicable are libraries developed from source species at significant distances from the target watersheds? That is, are specific ribotypes good indicators for only their source area or are they more like 'type' strains that are ubiquitous and useful within larger regions?

As suspected, analysis using the combined database yielded more source species identifications for the water sample isolates. It also changed the "profile" of source species, especially by increasing the significance of humans/septage as a source. This may have resulted from the nature of the human source species profiles included from New Hampshire, which were mostly from wastewater treatment facilities (WWTF), whereas the VT isolates were all from septage. The occurrence of contamination from leaky sewage pipes and illicit connections into storm drains has been well documented in NH Seacoast surface waters. Similar conditions may exist in parts of the VT watersheds. In any case, use of NH WWTF isolates proved useful for the VT study. The other most striking change was the many source species identified that were not even part of the VT library. The extent of the occurrence of coyotes in the VT watersheds is unknown, but fecal samples have been observed and sampled in Seacoast NH. Deer are suspected to be present in the VT study area, and apparently may be relatively important sources of *E. coli* in water. The experience from scat/fecal sampling in New Hampshire also suggests that exact speciation of the origin of the scat, in the absence of direct observation, can be tricky. In the final analysis, as reflected in the present study, it is the distinguishing between types of sources that will be most instructive to managers. Thus, if the significance of 'wildlife' in comparison to humans and pets can be determined, then managers can take appropriate actions. The addition of the source species profiles from this study to the larger database will also enhance future ribotyping studies conducted in the New England region.

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# Table 1. Sampling station descriptions.

## Colchester area watershed

| Site | Matrix | Name | Description |
|---|---|---|---|
| C2 | brook | Crooked Creek mouth | In-stream; mouth of Crooked Creek |
| C4 | stream | 60 Lakeshore Dr. | In-stream/stormdrain outfall |
| C5 | stream | Smith Hollow-mouth | In-stream; at mouth of Smith Hollow Creek |
| C7 | lake | Bayside Beach | Malletts Bay @ Bayside (public) beach |
| C8 | stream | The moorings | Malletts Bay, stormdrain outlet at marina |
| C9 | lake | Mills Point east | Outer Malletts Bay, at beach on east side of Mills Pt. |
| C12 | river | Winooski River | near mouth, at Fish & Wildlife access |
| C13 | stream | Sunderland Brook | at Pines I. Rd. crossing |

## Winooski area watershed

| Site | Matrix | Name | Description |
|---|---|---|---|
| W1 | brook | Morehouse Brook | In-stream; just above stormdrain outfall at Malletts Bay Ave. |
| W2 | stormdrain | Morehouse storm drain | Stormdrain outlet to Morehouse Brook at Malletts Bay Ave. |
| W6 | stormdrain | Canoe access | Stormdrain at canoe access near salmon hole |
| W8 | stormdrain | Hoods St. RR Xing | Stormdrain outfall to Winooski R. near old IGA bldg. |
| W11 | river | Salmon hole | In-river; salmon hole, Winooski side off of beach |

# Table 2. Sampling date and timing description.

| | Winooski watershed | | Colchester watershed | |
|---|---|---|---|---|
| Date | Weather | sample period | Weather | sample period |
| 8/22/00 | no rain | 8:20 to 10:50 | no rain | 9:25 to 11:45 |
| 8/23/00 | storm | 11:45 to 13:20 | storm | 10:20 to 13:40 |
| 8/29/00 | no rain | 8:50 to 9:57 | no rain | 10:11 to 11:47 |

**Table 3. Recovery success for *E. coli* isolates from known source species.**

| Species | # isolates received | # isolates ribotyped | % recovery |
|---|---|---|---|
| Cat | 25 | 20 | 80 |
| Cow | 50 | 41 | 82 |
| Dog | 22 | 14 | 64 |
| Horse | 20 | 11 | 55 |
| Mallard | 25 | 23 | 92 |
| Pigeon | 10 | 8 | 80 |
| Raccoon | 35 | 33 | 94 |
| Seagull | 35 | 32 | 91 |
| Septic/septage | 39 | 27 | 69 |
| TOTAL | 261 | 209 | 80 |

## Table 4. Ribotype similarity analysis results.

| Sample # 16xxx | rep | | Best- Fit >80% | >85% | >90% | 95-100% | % similarity | # of bands unknown | source |
|---|---|---|---|---|---|---|---|---|---|
| | | | **8/22/00** | | | | | | |
| 863 | C | c5 | gull | | | | 80 | 5 | 5 |
| | E | c5 | mallard | | | | 80 | 3 | 2 |
| 866 | A | c8 | cow-s | | | | 80 | 5 | 5 |
| 871 | D | c13 | raccoon | | | | 80 | 5 | 5 |
| 872 | D | w1 | gull | gull | gull | gull | 100 | 2 | 2 |
| 873 | B | w2 | gull | gull | | | 85.7 | 3 | 4 |
| 876 | B | w6 | septage | | | | 80 | 6 | 4 |
| | C | w6 | cat | | | | 81.8 | 11 | 11 |
| | E | w6 | cow | cow | cow | cow | 100 | 3 | 3 |
| 877 | C | w11 | raccoon | | | | 80 | 3 | 2 |
| | D | w11 | pigeon | pigeon | pigeon | pigeon | 100 | 2 | 2 |
| 878 | A | w8 | raccoon | raccoon | | | 88.9 | 4 | 5 |
| | E | w8 | cat | | | | 80 | 9 | 11 |
| | | | **8/23/00** | | | | | | |
| 887 | A | c2 | gull | | | | 80 | 6 | 9 |
| | C | c2 | gull | gull | | | 85.7 | 4 | 3 |
| | D | c2 | gull | gull | | | 85.7 | 4 | 3 |
| | E | c2 | cow | cow | cow | cow | 100 | 5 | 5 |
| 890 | A | c4 | cat | | | | 80 | 9 | 11 |
| | B | c4 | gull | gull | | | 85.7 | 4 | 3 |
| | E | c4 | raccoon | raccoon | raccoon | raccoon | 100 | 3 | 3 |
| 891 | A | c5 | gull | | | | 81.8 | 10 | 12 |
| | D | c5 | mallard | mallard | mallard | | 93.3 | 7 | 8 |
| | E | c5 | cat | cat | cat | cat | 100 | 3 | 3 |
| 894 | B | c7 | gull | gull | | | 85.7 | 9 | 12 |
| 900 | A | w6ff | raccoon | raccoon | raccoon | | 90.9 | 5 | 6 |
| 901 | E | w6m | mallard | mallard | mallard | mallard | 100 | 2 | 2 |
| 905 | A | w1ff | mallard | mallard | mallard | mallard | 100 | 2 | 2 |
| | B | w1ff | gull | gull | gull | gull | 100 | 2 | 2 |
| 907 | C | w2ff | horse | horse | horse | horse | 100 | 2 | 2 |
| | E | w2ff | raccoon | | | | 80 | 2 | 3 |
| 910 | B | w8m | cat | cat | | | 85.7 | 3 | 4 |
| | | | **8/29/00** | | | | | | |
| 937 | B | c4 | raccoon | raccoon | raccoon | raccoon | 100 | 2 | 2 |
| | C | c4 | raccoon | | | | 80 | 5 | 5 |
| 938 | A | c5 | cow | cow | cow | cow | 100 | 2 | 2 |
| | B | c5 | septage | septage | septage | septage | 100 | 3 | 3 |
| 940 | C | c7 | cat | cat | cat | cat | 100 | 3 | 3 |
| | E | c7 | dog | | | | 80 | 3 | 2 |
| dup | A | c7 | cow | | | | 80 | 3 | 2 |
| 948 | A | w2 | cow | | | | 80 | 5 | 5 |
| | D | w2 | raccoon | | | | 80 | 3 | 2 |
| 950 | B | w6 | septage | septage | | | 85.7 | 4 | 3 |
| 951 | A | w11 | mallard | mallard | mallard | mallard | 100 | 2 | 2 |
| | B | w11 | gull | | | | 80 | 3 | 2 |
| | D | w11 | cow | cow | cow | cow | 100 | 2 | 2 |
| Total | 44 | | 44 | 26 | 18 | 16 | Average= | 89 | 4.2 | 4.3 |

**Table 5a. Ribotyping/Dice analysis success for isolates from two Vermont watersheds: August, 2000.**

| Date | 8/22/00 | | | | 8/23/00 | | | | 8/29/00 | | | | TOTAL ISOLATES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | |
| Site | Rec'd* | R-typed | >80% | UID | Rec'd | R-typed | >80% | UID | Rec'd | R-typed | >80% | UID | Rec'd | R-typed | >80% | UID |
| **Colchester area watershed** | | | | | | | | | | | | | | | | |
| C2 | 5 | 1 | 0 | 1 | 5 | 5 | 4 | 1 | 5 | 5 | 0 | 5 | 15 | 11 | 4 | 7 |
| C4 | | | | | 5 | 5 | 3 | 2 | 5 | 4 | 2 | 2 | 10 | 9 | 5 | 4 |
| C5 | 5 | 3 | 2 | 1 | 5 | 5 | 3 | 2 | 5 | 5 | 2 | 3 | 15 | 13 | 7 | 6 |
| C7 | | | | | 5 | 4 | 1 | 3 | 10 | 8 | 3 | 5 | 15 | 12 | 4 | 8 |
| C8 | 5 | 4 | 1 | 3 | 5 | 3 | 0 | 3 | | | | | 10 | 7 | 1 | 6 |
| C9 | 1 | 1 | 0 | 1 | | | | | | | | | 1 | 1 | 0 | 1 |
| C12 | | | | | 1 | 1 | 0 | 1 | 7 | 3 | 0 | 3 | 8 | 4 | 0 | 4 |
| C13 | 5 | 5 | 1 | 4 | 5 | 3 | 0 | 3 | 5 | 5 | 0 | 5 | 15 | 13 | 1 | 12 |
| | | | | | | | | | | | | Total: | 89 | 70 | 22 | 48 |
| **Winooski area watershed** | | | | | | | | | | | | | | | | |
| W1 | 5 | 4 | 1 | 3 | 10 | 5 | 2 | 3 | | | | | 15 | 9 | 3 | 6 |
| W2 | 5 | 4 | 1 | 3 | 10 | 8 | 2 | 6 | 5 | 5 | 2 | 3 | 20 | 17 | 5 | 12 |
| W6 | 5 | 5 | 3 | 2 | 10 | 4 | 2 | 2 | 4 | 2 | 1 | 1 | 19 | 11 | 6 | 5 |
| W8 | 5 | 4 | 2 | 2 | 10 | 8 | 1 | 7 | 5 | 4 | 0 | 4 | 20 | 16 | 3 | 13 |
| W11 | 5 | 5 | 2 | 3 | | | | | 4 | 4 | 3 | 1 | 9 | 9 | 5 | 4 |
| | | | | | | | | | | | | Total: | 83 | 62 | 22 | 40 |
| | | | | | | | | | | | | **OVERALL TOTALS:** | **172** | **132** | **44** | **88** |

***Rec'd** = isolates received; **R-typed** = isolates ribotyped (>1 band); **>80%** = isolates with >80% identity to known isolate;
**UID** = Unidentified; isolates with <80% identity to any known isolates

**Table 5b. Ribotyping/Dice analysis success for isolates from two Vermont watersheds: August, 2000.**

| Date | 8/22/00 | | | | 8/23/00 | | | | 8/29/00 | | | | TOTAL ISOLATES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | |
| Site | Rec'd* | R-typed | >85% | UID | Rec'd | R-typed | >85% | UID | Rec'd | R-typed | >85% | UID | Rec'd | R-typed | >85% | UID |
| **Colchester area watershed** | | | | | | | | | | | | | | | | |
| C2 | 5 | 1 | 0 | 1 | 5 | 5 | 3 | 2 | 5 | 5 | 0 | 5 | 15 | 11 | 3 | 8 |
| C4 | | | | | 5 | 5 | 2 | 3 | 5 | 4 | 1 | 3 | 10 | 9 | 3 | 6 |
| C5 | 5 | 3 | 0 | 3 | 5 | 5 | 2 | 3 | 5 | 5 | 2 | 3 | 15 | 13 | 4 | 9 |
| C7 | | | | | 5 | 4 | 1 | 3 | 10 | 8 | 1 | 7 | 15 | 12 | 2 | 10 |
| C8 | 5 | 4 | 0 | 4 | 5 | 3 | 0 | 3 | | | | | 10 | 7 | 0 | 7 |
| C9 | 1 | 1 | 0 | 1 | | | | | | | | | 1 | 1 | 0 | 1 |
| C12 | | | | | 1 | 1 | 0 | 1 | 7 | 3 | 0 | 3 | 8 | 4 | 0 | 4 |
| C13 | 5 | 5 | 0 | 5 | 5 | 3 | 0 | 3 | 5 | 5 | 0 | 5 | 15 | 13 | 0 | 13 |
| | | | | | | | | | | | | Total: | 89 | 70 | 12 | 58 |
| **Winooski area watershed** | | | | | | | | | | | | | | | | |
| W1 | 5 | 4 | 1 | 3 | 10 | 5 | 2 | 3 | | | | | 15 | 9 | 3 | 6 |
| W2 | 5 | 4 | 1 | 3 | 10 | 8 | 1 | 7 | 5 | 5 | 0 | 5 | 20 | 17 | 2 | 15 |
| W6 | 5 | 5 | 1 | 4 | 10 | 4 | 2 | 2 | 4 | 2 | 1 | 1 | 19 | 11 | 4 | 7 |
| W8 | 5 | 4 | 1 | 3 | 10 | 8 | 1 | 7 | 5 | 4 | 0 | 4 | 20 | 16 | 2 | 14 |
| W11 | 5 | 5 | 1 | 4 | | | | | 4 | 4 | 2 | 2 | 9 | 9 | 3 | 6 |
| | | | | | | | | | | | | Total: | 83 | 62 | 14 | 48 |
| | | | | | | | | | | | | **OVERALL TOTALS:** | **172** | **132** | **26** | **106** |

***Rec'd** = isolates received; **R-typed** = isolates ribotyped (>1 band); **>85%** = isolates with >85% identity to known isolate;
**UID** = Unidentified; isolates with <85% identity to any known isolates

**Table 5c. Ribotyping/Dice analysis success for isolates from two Vermont watersheds: August, 2000.**

| Date | 8/22/00 | | | | 8/23/00 | | | | 8/29/00 | | | | TOTAL ISOLATES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | |
| Site | Rec'd* | R-typed | >90% | UID | Rec'd | R-typed | >90% | UID | Rec'd | R-typed | >90% | UID | Rec'd | R-typed | >90% | UID |
| **Colchester area watershed** | | | | | | | | | | | | | | | | |
| C2 | 5 | 1 | 0 | 1 | 5 | 5 | 1 | 4 | 5 | 5 | 0 | 5 | 15 | 11 | 1 | 10 |
| C4 | | | | | 5 | 5 | 1 | 4 | 5 | 4 | 1 | 3 | 10 | 9 | 2 | 7 |
| C5 | 5 | 3 | 0 | 3 | 5 | 5 | 2 | 3 | 5 | 5 | 2 | 3 | 15 | 13 | 4 | 9 |
| C7 | | | | | 5 | 4 | 0 | 4 | 10 | 8 | 1 | 7 | 15 | 12 | 1 | 11 |
| C8 | 5 | 4 | 0 | 4 | 5 | 3 | 0 | 3 | | | | | 10 | 7 | 0 | 7 |
| C9 | 1 | 1 | 0 | 1 | | | | | | | | | 1 | 1 | 0 | 1 |
| C12 | | | | | 1 | 1 | 0 | 1 | 7 | 3 | 0 | 3 | 8 | 4 | 0 | 4 |
| C13 | 5 | 5 | 0 | 5 | 5 | 3 | 0 | 3 | 5 | 5 | 0 | 5 | 15 | 13 | 0 | 13 |
| | | | | | | | | | | | | Total: | 89 | 70 | 8 | 62 |
| **Winooski area watershed** | | | | | | | | | | | | | | | | |
| W1 | 5 | 4 | 1 | 3 | 10 | 5 | 2 | 3 | | | | | 15 | 9 | 3 | 6 |
| W2 | 5 | 4 | 0 | 4 | 10 | 8 | 1 | 7 | 5 | 5 | 0 | 5 | 20 | 17 | 1 | 16 |
| W6 | 5 | 5 | 1 | 4 | 10 | 4 | 2 | 2 | 4 | 2 | 0 | 2 | 19 | 11 | 3 | 8 |
| W8 | 5 | 4 | 0 | 4 | 10 | 8 | 0 | 8 | 5 | 4 | 0 | 4 | 20 | 16 | 0 | 16 |
| W11 | 5 | 5 | 1 | 4 | | | | | 4 | 4 | 2 | 2 | 9 | 9 | 3 | 6 |
| | | | | | | | | | | | | Total: | 83 | 62 | 10 | 52 |
| | | | | | | | | | | | | **OVERALL TOTALS:** | **172** | **132** | **18** | **114** |

***Rec'd** = isolates received; **R-typed** = isolates ribotyped (>1 band); **>90%** = isolates with >90% identity to known isolate;
**UID** = Unidentified; isolates with <90% identity to any known isolates

**Table 6a. Source species for *E. coli* isolated from two Vermont watersheds during 3 days in August, 2000.**

***Dice Analysis Results: ≥ 80% Matching Similarity***

| Site | Total isolates | % Unidentified | % Source species | | | | | | | | | % Species Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cats | Cows | Septage | Seagulls | Mallards | Raccoons | Horses | Pigeons | Dogs | |
| **Colchester watershed** | | | | | | | | | | | | |
| C2 | 11 | 64 | | 9 | | 27 | | | | | | 36 |
| C4 | 9 | 44 | 11 | | | 11 | | 33 | | | | 56 |
| C5 | 13 | 46 | 8 | 8 | 8 | 15 | 15 | | | | | 54 |
| C7 | 12 | 67 | 8 | 8 | | 8 | | | | | 8 | 33 |
| C8 | 7 | 86 | | 14 | | | | | | | | 14 |
| C9 | 1 | 100 | | | | | | | | | | 0 |
| C12 | 4 | 100 | | | | | | | | | | 0 |
| C13 | 13 | 92 | | | | | | 8 | | | | 8 |
| **Winooski watershed** | | | | | | | | | | | | |
| W1 | 9 | 67 | | | | 15 | 8 | | | | | 33 |
| W2 | 17 | 71 | | 6 | | 6 | | 12 | 6 | | | 29 |
| W6 | 11 | 45 | 9 | 9 | 18 | | 9 | 9 | | | | 55 |
| W8 | 16 | 81 | 13 | | | | | 6 | | | | 19 |
| W11 | 9 | 44 | | 11 | | 11 | 11 | 11 | | 11 | | 56 |

**Table 6b. Source species for *E. coli* isolated from two Vermont watersheds during 3 days in August, 2000.**

## *Dice Analysis Results: >85% Matching Similarity*

| Site | Total isolates | % Unidentified | % Source species | | | | | | | | | % Species Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cats | Cows | Septage | Seagulls | Mallards | Raccoons | Horses | Pigeons | Dogs | |
| **Colchester watershed** | | | | | | | | | | | | |
| C2 | 11 | 73 | | 9 | | 18 | | | | | | 27 |
| C4 | 9 | 67 | | | | 11 | | 22 | | | | 33 |
| C5 | 13 | 69 | 8 | 8 | 8 | | 8 | | | | | 31 |
| C7 | 12 | 83 | 8 | | | 8 | | | | | | 17 |
| C8 | 7 | 100 | | | | | | | | | | 0 |
| C9 | 1 | 100 | | | | | | | | | | 0 |
| C12 | 4 | 100 | | | | | | | | | | 0 |
| C13 | 13 | 100 | | | | | | | | | | 0 |
| **Winooski watershed** | | | | | | | | | | | | |
| W1 | 9 | 67 | | | | 15 | 8 | | | | | 33 |
| W2 | 17 | 88 | | | | 6 | | | 6 | | | 12 |
| W6 | 11 | 64 | | 9 | 9 | | 9 | 9 | | | | 36 |
| W8 | 16 | 88 | 6 | | | | | 6 | | | | 13 |
| W11 | 9 | 67 | | 11 | | | 11 | | | 11 | | 33 |

**Table 6c. Source species for *E. coli* isolated from two Vermont watersheds during 3 days in August, 2000.**

***Dice Analysis Results: >90% Matching Similarity***

| Site | Total isolates | % Unidentified | % Source species | | | | | | | | | % Species Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cats | Cows | Septage | Seagulls | Mallards | Raccoons | Horses | Pigeons | Dogs | |
| **Colchester watershed** | | | | | | | | | | | | |
| C2 | 11 | 91 | | 9 | | | | | | | | 9 |
| C4 | 9 | 78 | | | | | | 22 | | | | 22 |
| C5 | 13 | 69 | 8 | 8 | 8 | | 8 | | | | | 31 |
| C7 | 12 | 92 | 8 | | | | | | | | | 8 |
| C8 | 7 | 100 | | | | | | | | | | 0 |
| C9 | 1 | 100 | | | | | | | | | | 0 |
| C12 | 4 | 100 | | | | | | | | | | 0 |
| C13 | 13 | 100 | | | | | | | | | | 0 |
| **Winooski watershed** | | | | | | | | | | | | |
| W1 | 9 | 67 | | | | 22 | 11 | | | | | 33 |
| W2 | 17 | 94 | | | | | | | 6 | | | 6 |
| W6 | 11 | 73 | | 9 | | | 9 | 9 | | | | 27 |
| W8 | 16 | 100 | | | | | | | | | | 0 |
| W11 | 9 | 67 | | 11 | | | 11 | | | 11 | | 33 |

**Table 7a. Isolate occurrence from each source species in the 2 watersheds.**

| Source species | **80% similarity** | # of isolates | | |
|---|---|---|---|---|
| | | Colc. | Win. | Total |
| Cat | | 3 | 3 | **6** |
| Cow | | 4 | 3 | **7** |
| Dog | | 1 | 0 | **1** |
| Horse | | 0 | 1 | **1** |
| Mallard | | 2 | 3 | **5** |
| Pigeon | | 0 | 1 | **1** |
| Raccoon | | 4 | 5 | **9** |
| Seagull | | 7 | 4 | **11** |
| Septic/septage | | 1 | 2 | **3** |
| Total | | **22** | **22** | **44** |

**Table 7b. Isolate occurrence from each source species in the 2 watersheds.**

| Source species | **85% similarity** | # of isolates | | |
|---|---|---|---|---|
| | | Colc. | Win. | Total |
| Cat | | 2 | 1 | **3** |
| Cow | | 2 | 2 | **4** |
| Dog | | 0 | 0 | **0** |
| Horse | | 0 | 1 | **1** |
| Mallard | | 1 | 3 | **4** |
| Pigeon | | 0 | 1 | **1** |
| Raccoon | | 2 | 2 | **4** |
| Seagull | | 4 | 3 | **7** |
| Septic/septage | | 1 | 1 | **2** |
| Total | | **12** | **14** | **26** |

**Table 7c. Isolate occurrence from each source species in the 2 watersheds.**

| Source species | **90% similarity** | # of isolates | | |
|---|---|---|---|---|
| | | Colc. | Win. | Total |
| Cat | | 2 | 0 | **2** |
| Cow | | 2 | 2 | **4** |
| Dog | | 0 | 0 | **0** |
| Horse | | 0 | 1 | **1** |
| Mallard | | 1 | 3 | **4** |
| Pigeon | | 0 | 1 | **1** |
| Raccoon | | 2 | 1 | **3** |
| Seagull | | 0 | 2 | **2** |
| Septic/septage | | 1 | 0 | **1** |
| Total | | **8** | **10** | **18** |

**Table 8a. Occurrences for identified source species at the 13 sampling stations on the four different sampling times.**
*≥ 80% Matching Similarity*

| Site | Total sample times | Source species | | | | | Repeat source sp. |
|---|---|---|---|---|---|---|---|
| | | 8/22/00 | 8/23/00 | | | 8/29/00 | |
| | | | post | "FF" | mid-storm | | |
| Colchester | | | | | | | |
| 2 | 3 | | gull, cow | NS | NS | | |
| 4 | 2 | NS* | raccoon, gull, cat | NS | NS | raccoon | raccoon |
| 5 | 3 | gull, mallard | gull, mallard, cat | NS | NS | cow , septage | gull, mallard |
| 7 | 2 | NS | gull | NS | NS | cat, dog, cow | |
| 8 | 2 | cow | | NS | NS | NS | |
| 9 | 1 | | NS | NS | NS | NS | |
| 12 | 2 | NS | | NS | NS | | |
| 13 | 3 | racoon | | NS | NS | | |
| Winooski | | | | | | | |
| 1 | 3 | gull | NS | gull, mallard | | NS | gull |
| 2 | 4 | gull | NS | horse, raccoon | | cow , raccoon | raccoon |
| 6 | 4 | septage, cat, cow | NS | raccoon | mallard | septage | septage |
| 8 | 4 | raccoon, cat | NS | | cat | | cat |
| 11 | 2 | raccoon, pigeon | NS | NS | NS | gull, mallard, cow | |

*NS: No sample collected.

**Table 8b. Occurrences for identified source species at the 13 sampling stations on the four different sampling times.**

*>85% Matching Similarity*

| Site | Total sample times | Source species | | | | | Repeat source sp. |
|---|---|---|---|---|---|---|---|
| | | 8/22/00 | 8/23/00 | | | 8/29/00 | |
| | | | post | "FF" | mid-storm | | |
| Colchester | | | | | | | |
| 2 | 3 | | gull, cow | NS | NS | | |
| 4 | 2 | NS* | raccoon, gull | NS | NS | raccoon | raccoon |
| 5 | 3 | | mallard, cat | NS | NS | cow , septage | |
| 7 | 2 | NS | gull | NS | NS | cat | |
| 8 | 2 | | | NS | NS | NS | |
| 9 | 1 | | NS | NS | NS | NS | |
| 12 | 2 | NS | | NS | NS | | |
| 13 | 3 | | | NS | NS | | |
| Winooski | | | | | | | |
| 1 | 3 | gull | NS | gull, mallard | | NS | gull |
| 2 | 4 | gull | NS | horse | | | |
| 6 | 4 | cow | NS | raccoon | mallard | septage | |
| 8 | 4 | raccoon | NS | | cat | | |
| 11 | 2 | raccoon, pigeon | NS | NS | NS | mallard, cow | |

*NS: No sample collected.

†Septage occurred as source for both sample times on 8/23/00.

**Table 8c. Occurrences for identified source species at the 13 sampling stations on the four different sampling times.**
*>90% Matching Similarity*

| Site | Total sample times | Source species | | | | | Repeat source sp. |
|---|---|---|---|---|---|---|---|
| | | 8/22/00 | 8/23/00 | | | 8/29/00 | |
| | | | post | "FF" | mid-storm | | |
| Colchester | | | | | | | |
| 2 | 3 | | cow | NS | NS | | |
| 4 | 2 | NS* | | NS | NS | raccoon | |
| 5 | 3 | | mallard, cat | NS | NS | cow †, septage | |
| 7 | 2 | NS | | NS | NS | cat | |
| 8 | 2 | | | NS | NS | NS | |
| 9 | 1 | | NS | NS | NS | NS | |
| 12 | 2 | NS | | NS | NS | | |
| 13 | 3 | | | NS | NS | | |
| Winooski | | | | | | | |
| 1 | 3 | gull | NS | gull, mallard | | NS | gull |
| 2 | 4 | | NS | horse | | | |
| 6 | 4 | cow | NS | raccoon | mallard | | |
| 8 | 4 | | NS | | | | |
| 11 | 2 | pigeon | NS | NS | NS | mallard, cow | |

*NS: No sample collected.
†Septage occurred as source for both sample times on 8/23/00.

# Table 9. Intraspecies comparisons of ribotype profiles.

| Source species | # of profiles | Matching profiles | # bands per profile | # profiles per match | Clone/isolate ratio (%) |
|---|---|---|---|---|---|
| cow-s | 19 | 1 | 4 | 2 | 95 |
| cow-f | 17 | 2 | 2 for both | 2 | 88 |
| cows | 36 | 3 | 2 or 4 | 2 | 92 |
| cat | 18 | 0 | | | 100 |
| horse | 9 | 2 | 2 for both | 2 or 3 | 67 |
| raccoon | 31 | 1 | 2 | 3 | 94 |
| pigeon | 7 | 0 | | | 100 |
| mallard | 22 | 3 | 2, 4, or 8 | 2 for all 3 | 86 |
| dog | 14 | 2 | 4 for both | 2 for both | 86 |
| seagull | 30 | 1 | 3 | 2 | 97 |
| septage | 19 | 0 | | | 100 |
| Totals | 186 | 12 | | Average | 91 |

## Table 10. Interspecies comparisons and summary of matching ribotype profiles.

| # source species per match | # of profiles per match | # of bands in matched profiles | Source species included in match |
|---|---|---|---|
| 3 | 5 | 2 | mallard, raccoon, gull |
| 2 | 2 | 3 | raccoon, gull |
| 2 | 2 | 3 | mallard, raccoon, gull |
| 2 | 2 | 4 | cat, septage |
| 2 | 2 | 2 | horse, dog |
| 2 | 4 | 2 | mallard, horse |
| 3 | 4 | 2 | cow , mallard, gull |

# Table 11. Ribotype similarity analysis results for all water sample profiles with >3 bands.

| Site name | Site # | Best- Fit >80% | >85% | >90% | 95-100% | % similarity | # of bands unknown | source |
|---|---|---|---|---|---|---|---|---|
| | | **8/22/00** | | | | | | |
| Smith Hollow | c5 | gull | | | | 80 | 5 | 5 |
| The moorings | c8 | cow | | | | 80 | 5 | 5 |
| Sunderland Bk. | c13 | raccoon | | | | 80 | 5 | 5 |
| Canoe access | w6 | septage | | | | 80 | 6 | 4 |
| | | cat | | | | 81.8 | 11 | 11 |
| Hoods St. | w8 | raccoon | raccoon | | | 88.9 | 4 | 5 |
| RR Xing | | cat | | | | 80 | 9 | 11 |
| | | **8/23/00** | | | | | | |
| Crooked Creek | c2 | gull | | | | 80 | 6 | 9 |
| mouth | | gull | gull | | | 85.7 | 4 | 3 |
| | | gull | gull | | | 85.7 | 4 | 3 |
| | | cow | cow | cow | cow | 100 | 5 | 5 |
| 60 Lakeshore Dr. | c4 | cat | | | | 80 | 9 | 11 |
| | | gull | gull | | | 85.7 | 4 | 3 |
| Smith Hollow | c5 | gull | | | | 81.8 | 10 | 12 |
| | | mallard | mallard | mallard | | 93.3 | 7 | 8 |
| Bayside Beach | c7 | gull | gull | | | 85.7 | 9 | 12 |
| Canoe access | w6ff | raccoon | raccoon | raccoon | | 90.9 | 5 | 6 |
| | | **8/29/00** | | | | | | |
| 60 Lakeshore Dr. | c4 | raccoon | | | | 80 | 5 | 5 |
| Morehouse drain | w2 | cow | | | | 80 | 5 | 5 |
| Canoe access | w6 | septage | septage | | | 85.7 | 4 | 3 |
| Total | | 20 | 9 | 3 | 1 | Average= 84 | 6.1 | 6.6 |

**Table 12. Ribotyping/Dice analysis success for isolates with >3 bands from 2 Vermont watersheds: 8/02.**

| Date | 8/22/00 | | | | 8/23/00 | | | | 8/29/00 | | | | TOTAL ISOLATES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # of Isolates | | | | # of Isolates | | | | # of Isolates | | | | # of Isolates | | | |
| Site | Rec'd* | R-typed | >80% | UID | Rec'd | R-typed | >80% | UID | Rec'd | R-typed | >80% | UID | Rec'd | R-typed | >80% | UID |
| **Colchester area watershed** | | | | | | | | | | | | | | | | |
| C2 | 5 | 1 | 0 | 1 | 5 | 4 | 4 | 0 | 5 | 2 | 0 | 2 | 15 | 7 | 4 | 3 |
| C4 | | | | | 5 | 3 | 2 | 1 | 5 | 1 | 1 | 0 | 10 | 4 | 3 | 1 |
| C5 | 5 | 1 | 1 | 0 | 5 | 4 | 2 | 2 | 5 | 0 | | | 15 | 5 | 3 | 2 |
| C7 | | | | | 5 | 4 | 1 | 3 | 10 | 2 | 0 | 2 | 15 | 6 | 1 | 5 |
| C8 | 5 | 4 | 1 | 3 | 5 | 1 | 0 | 1 | | | | | 10 | 5 | 1 | 4 |
| C9 | 1 | 0 | | | | | | | | | | | 1 | 0 | 0 | 0 |
| C12 | | | | | 1 | 1 | 0 | 1 | 7 | 3 | 0 | 3 | 8 | 4 | 0 | 4 |
| C13 | 5 | 3 | 1 | 2 | 5 | 2 | 0 | 2 | 5 | 4 | 0 | 4 | 15 | 9 | 1 | 8 |
| | | | | | | | | | | | | Total: | 89 | 40 | 13 | 27 |
| **Winooski area watershed** | | | | | | | | | | | | | | | | |
| W1 | 5 | 2 | 0 | 2 | 10 | 0 | | | | | | | 15 | 2 | 0 | 2 |
| W2 | 5 | 1 | 0 | 1 | 10 | 1 | 0 | 1 | 5 | 4 | 1 | 3 | 20 | 6 | 1 | 5 |
| W6 | 5 | 4 | 2 | 2 | 10 | 3 | 1 | 2 | 4 | 1 | 1 | 0 | 19 | 8 | 4 | 4 |
| W8 | 5 | 4 | 2 | 2 | 10 | 1 | 0 | 1 | 5 | 4 | 0 | 4 | 20 | 9 | 2 | 7 |
| W11 | 5 | 1 | 0 | 1 | | | | | 4 | 0 | | | 9 | 1 | 0 | 1 |
| | | | | | | | | | | | | Total: | 83 | 26 | 7 | 19 |
| | | | | | | | | | | | | **OVERALL TOTALS:** | **172** | **66** | **20** | **46** |

***Rec'd** = isolates received; **R-typed** = isolates ribotyped (>3 bands); **>80%** = isolates with >80% identity to known isolate;
**UID** = Unidentified; isolates with <80% identity to any known isolates

**Table 13. Source species for *E. coli* (>3 bands in profile) isolated from 2 Vermont watersheds during 3 days in August, 2000.** (Dice Analysis Results: >80% Matching Similarity)

| Site | Total isolates | % Unidentified | % Source species | | | | | | | | | % Species Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cats | Cows | Septage | Seagulls | Mallards | Raccoons | Horses | Pigeons | Dogs | |
| **Colchester watershed** | | | | | | | | | | | | |
| C2 | 7 | 43 | | 14 | | 43 | | | | | | 57 |
| C4 | 4 | 25 | 25 | | | 25 | | 25 | | | | 75 |
| C5 | 5 | 40 | | | | 40 | 20 | | | | | 60 |
| C7 | 6 | 83 | | | | 17 | | | | | | 17 |
| C8 | 5 | 80 | | 20 | | | | | | | | 20 |
| C9 | 0 | 100 | | | | | | | | | | 0 |
| C12 | 4 | 100 | | | | | | | | | | 0 |
| C13 | 9 | 89 | | | | | | 11 | | | | 11 |
| Totals | 40 | 68 | 3 | 5 | | 18 | 3 | 5 | | | | 33 |
| **Winooski watershed** | | | | | | | | | | | | |
| W1 | 2 | 100 | | | | | | | | | | 0 |
| W2 | 6 | 83 | | 17 | | | | | | | | 17 |
| W6 | 8 | 50 | 13 | | 25 | | | 13 | | | | 50 |
| W8 | 9 | 78 | 11 | | | | | 11 | | | | 22 |
| W11 | 1 | 100 | | | | | | | | | | 0 |
| Totals | 26 | 73 | 8 | 4 | 8 | | | 8 | | | | 27 |
| **OVERALL TOTALS** | **66** | **70** | **5** | **5** | **3** | **11** | **2** | **6** | | | | **30** |

**Table 14. Isolate occurrence from each source species in the 2 watersheds.** Profiles used all had >3 bands, matches had >80% similarity.

| Source species | # of isolates | | |
|---|---|---|---|
| | Colc. | Win. | Total |
| Cat | 1 | 2 | **3** |
| Cow | 2 | 1 | **3** |
| Dog | 0 | 0 | **0** |
| Horse | 0 | 0 | **0** |
| Mallard | 1 | 0 | **1** |
| Pigeon | 0 | 0 | **0** |
| Raccoon | 2 | 2 | **4** |
| Seagull | 7 | 0 | **7** |
| Septic/septage | 0 | 2 | **2** |
| Total | **13** | **7** | **20** |

**Table 15. Recovery success for *E. coli* isolates from known source species.**

| Species | # isolates received | # isolates ribotyped | % recovery | # isolates w/ >3 bands | % isolates w/ >3 bands | % RT'd isolates w/ >3 bands |
|---|---|---|---|---|---|---|
| Cat | 25 | 20 | 80 | 16 | 64 | 80 |
| Cow | 50 | 41 | 82 | 26 | 52 | 63 |
| Dog | 22 | 14 | 64 | 8 | 36 | 57 |
| Horse | 20 | 11 | 55 | 1 | 5 | 9 |
| Mallard | 25 | 23 | 92 | 14 | 56 | 61 |
| Pigeon | 10 | 8 | 80 | 3 | 30 | 38 |
| Raccoon | 35 | 33 | 94 | 14 | 40 | 42 |
| Seagull | 35 | 32 | 91 | 17 | 49 | 53 |
| Sewage | 39 | 27 | 69 | 10 | 26 | 37 |
| TOTAL | 261 | 209 | 80 | 109 | 42 | 52 |

# Table 16. New Hampshire (1998-10/01) and NH+VT source species databases.

| Species | Total *E. coli* isolates-NH | Total *E. coli* ribotypes*-NH | % NH isolates as ribotypes | NH ribotypes >2 bands | VT ribotypes >2 bands | VT & NH ribotypes >2 bands |
|---|---|---|---|---|---|---|
| buffalo | 4 | 0 | 0% | 0 | 0 | 0 |
| cat | 8 | 3 | 38% | 2 | 18 | 20 |
| chicken | 3 | 3 | 100% | 2 | 0 | 2 |
| cormorant | 42 | 16 | 38% | 14 | 0 | 14 |
| cow | 14 | 8 | 57% | 6 | 29 | 35 |
| coyote | 58 | 7 | 12% | 6 | 0 | 6 |
| deer | 115 | 56 | 49% | 43 | 0 | 43 |
| dog | 20 | 10 | 50% | 9 | 10 | 19 |
| duck | 20 | 2 | 10% | 2 | 0 | 2 |
| geese | 40 | 21 | 53% | 19 | 0 | 19 |
| gull | 11 | 5 | 45% | 5 | 26 | 31 |
| horse | 24 | 15 | 63% | 14 | 3 | 17 |
| human/septage | 81 | 61 | 75% | 49 | 16 | 65 |
| mallard | 0 | - | - | 0 | 17 | 17 |
| muskrat | 12 | 6 | 50% | 5 | 0 | 5 |
| pigeon | 6 | 2 | 33% | 2 | 5 | 7 |
| rabbit | 4 | 0 | 0% | 0 | 0 | 0 |
| raccoon | 4 | 19 | 475% | 14 | 23 | 37 |
| red fox | 81 | 8 | 10% | 7 | 0 | 7 |
| robin | 4 | 3 | 75% | 3 | 0 | 3 |
| wild turkey | 7 | 0 | 0% | 0 | 0 | 0 |
| **Totals:** | **558** | **245** | **44%** | **202** | **147** | **349** |

*More ribotypes will eventually be included following further re-processing.

## Table 17a. Ribotype analysis of *E. coli* isolates from 2 VT watersheds using VT and VT+NH known source databases: 8/22/00.

| 8/22/00 | | | VT library | | | NH&VT-best fit ≥80% | | |
|---|---|---|---|---|---|---|---|---|
| Site name | Site # | Species | % similarity | # of bands unknown | # of bands source | Species | % similarity | bands source |
| Crooked Creek | c2 | dog | 66.7 | 5 | 4 | raccoon | 80 | 5 |
| Smith Hollow | c5 | gull | 80 | 5 | 5 | deer | 88.9 | |
| | | mallard | 80* | 3 | 2 | mallard | 80* | 2 |
| The moorings | c8 | cow | 80 | 5 | 5 | raccoon | 83.3 | 7 |
| | | gull | 73.5 | 7 | 12 | deer | 82.4 | 10 |
| | | gull | 18.2 | 8 | 3 | | | |
| | | septage | 66.7 | 6 | 6 | | | |
| Mills Pond-east | c9 | cow | 75 | 3 | 5 | | | |
| Sunderland Bk. | c13 | cat | 57.1 | 3 | 3 | | | |
| | | gull | 40 | 3 | 2 | | | |
| | | racoon | 76.9 | 6 | 7 | | | |
| | | racoon | 80 | 5 | 5 | human | 88.9 | 4 |
| | | septage | 66.7 | 7 | 5 | chicken | 80 | 8 |
| Morehouse Bk. | w1 | gull | 75 | 4 | 4 | human | 85.7 | 3 |
| | | mallard | 75 | 4 | 4 | human | 80 | 6 |
| Morehouse drain | w2 | gull | 85.7 | 3 | 4 | raccoon | 100 | 3 |
| | | septage | 61.5 | 8 | 5 | | | |
| Canoe access | w6 | racoon | 72.7 | 7 | 4 | | | 6 |
| | | septage | 80 | 6 | 4 | horse | 83.3 | 6 |
| | | cat | 81.8 | 11 | 11 | cat | 81.8 | 11 |
| | | mallard | 75 | 7 | 9 | deer | 85.7 | 7 |
| | | cow | 100 | 3 | 3 | cow | 100 | 3 |
| Salmon hole | w11 | racoon | 33.3 | 3 | 3 | deer | 100 | 3 |
| Winooski R. | | cow | 66.7 | 8 | 7 | dog | 80 | 7 |
| | | racoon | 80* | 3 | 2 | raccoon | 80* | 2 |
| | | racoon | 66.7 | 3 | 3 | red fox | 85.7 | 4 |
| Hoods St. | w8 | racoon | 88.9 | 4 | 5 | raccoon | 88.9 | 5 |
| RR Xing | | racoon | 75 | 4 | 4 | deer | 85.7 | 3 |
| | | gull | 58.8 | 9 | 8 | | | |
| | | cat | 80 | 9 | 11 | cat | 80 | 11 |
| **TOTALS** | | **30** | **#≥80% similarity: 9** | | | **19** | | |

*2 bands for source library isolate; not accepted.

## Table 17b. Ribotype analysis of *E. coli* isolates from 2 VT watersheds using VT and VT+NH known source databases: 8/23/00.

| 8/23/00 | | | VT library | | | NH&VT-best fit ≥80% | | |
|---|---|---|---|---|---|---|---|---|
| Site name | Site # | Species | % similarity | # of bands unknown | # of bands source | Species | % similarity | bands source |
| Crooked Creek mouth | c2 | gull | 80 | 6 | 9 | gull | 80 | 9 |
| | | cow | 88.9 | 4 | 5 | cow | 88.9 | 5 |
| | | gull | 85.7 | 4 | 3 | gull | 85.7 | 3 |
| | | cow | 100 | 5 | 5 | cow | 100 | 5 |
| 60 Lakeshore Dr. | c4 | cat | 80 | 9 | 11 | cat | 80 | 11 |
| | | gull | 85.7 | 4 | 3 | gull | 85.7 | 3 |
| | | gull | 66.7 | 6 | 3 | human | 93.3 | 7 |
| | | racoon | 100 | 3 | 3 | raccoon | 100 | 3 |
| Smith Hollow mouth | c5 | gull | 81.8 | 10 | 12 | coyote | 82.4 | 7 |
| | | gull | 66.7 | 5 | 5 | raccoon | 88.9 | 5 |
| | | gull | 69.6 | 11 | 12 | human | 80 | 9 |
| | | mallard | 93.3 | 7 | 8 | mallard | 93.3 | 8 |
| | | cat | 100 | 3 | 3 | cat | 100 | 3 |
| Bayside Beach | c7 | gull | 85.7 | 9 | 12 | deer | 87.5 | 7 |
| | | cow | 71.4 | 7 | 7 | | | |
| | | mallard | 60 | 8 | 8 | | | |
| | | cat | 60 | 7 | 3 | deer | 87.3 | 10 |
| The moorings | c8 | racoon | 57.1 | 4 | 3 | | | |
| | | cow | 40 | 3 | 4 | | | |
| Winooski River | c12 | mallard | 75 | 4 | 3 | | | |
| Sunderland Bk. | c13 | gull | 66.7 | 9 | 12 | human | 82.4 | 8 |
| | | cat | 70.6 | 6 | 11 | chicken | 85.7 | 8 |
| | | cow | 66.7 | 3 | 2 | muskrat | 89.7 | 4 |
| Canoe access | w6ff | racoon | 90.9 | 5 | 6 | raccoon | 90.9 | 6 |
| | | cat | 62.5 | 8 | 7 | human | 82.4 | 8 |
| | | gull | 66.7 | 5 | 8 | | | |
| Morehouse Bk | w1ff | cat | 75 | 3 | 5 | | | |
| | w1m | dog | 66.7 | 3 | 3 | | | |
| Morehouse drain | w2ff | racoon | 71.4 | 7 | 7 | goose | 83.3 | 5 |
| Hoods St. RR Xing | w8mid | racoon | 50 | 5 | 3 | | | |
| | | cat | 85.7 | 3 | 4 | cat | 85.7 | 4 |
| **TOTALS** | | **31** | **#≥80% similarity: 13** | | | **22** | | |

*2 bands for source library isolate; not accepted.

## Table 17c. Ribotype analysis of *E. coli* isolates from 2 VT watersheds using VT and VT+NH known source databases: 8/29/00.

| 8/29/00 | | | VT library | | | NH&VT-best fit ≥80% | | |
|---|---|---|---|---|---|---|---|---|
| Site name | Site # | Species | % similarity | # of bands unknown | # of bands source | Species | % similarity | bands source |
| Crooked Creek | c2 | gull | 66.7 | 5 | 7 | | | |
| | | racoon | 57.1 | 8 | 6 | | | |
| 60 Lakeshore Dr. | c4 | septage | 40 | 3 | 2 | | | |
| | | racoon | 80 | 5 | 5 | raccoon | 80 | 5 |
| | | racoon | 66.7 | 3 | 3 | | | |
| Smith Hollow | c5 | septage | 100 | 3 | 3 | septage | 100 | 3 |
| Bayside Beach | c7 | cat | 100 | 3 | 3 | cat | 100 | 3 |
| | | horse | 66.7 | 3 | 3 | deer | 85.7 | 4 |
| | | dog | 80* | 3 | 2 | deer | 85.7 | 4 |
| Winooski River | c12 | cow | 55.6 | 11 | 7 | | | |
| | | cat | 66.7 | 7 | 7 | goose | 85.7 | 6 |
| Sunderland Bk. | c13 | multiple | 25 | 6 | 1 to 2 | | | |
| | | gull | 62.5 | 8 | 8 | | | |
| | | racoon | 51.1 | 7 | 7 | | | |
| | | cow | 75 | 4 | 4 | | | |
| Morehouse stormdrain | w2 | cow | 80 | 5 | 5 | cow | 80 | 5 |
| | | cow | 68.7 | 4 | 2 | | | |
| | | racoon | 61.5 | 6 | 7 | | | |
| | | racoon | 80* | 3 | 2 | raccoon | 80* | 2 |
| | | racoon | 66.7 | 4 | 2 | | | |
| Canoe access | w6 | septage | 85.7 | 4 | 3 | septage | 85.7 | 3 |
| | | mallard | 33 | 3 | 3 | | | |
| Salmon hole Winooski R. | w11 | gull | 80* | 3 | 2 | gull | 80* | 2 |
| | | racoon | 66.7 | 3 | 3 | | | |
| 952 | w8 | cow | 50 | 4 | 7 | | | |
| | | racoon | 60 | 5 | 5 | | | |
| | | mallard | 66.7 | 6 | 4 | | | |
| | | cow | 50 | 4 | 4 | human | 85.5 | 3 |
| duplicates | c7 | cow | 80* | 3 | 2 | human | 80* | 2 |
| | | horse | 60 | 5 | 5 | | | |
| | | cow | 57.1 | 7 | 7 | | | |
| duplicate | c12 | gull | 72.2 | 6 | 5 | | | |
| **TOTALS** | | **32** | **#≥80% similarity: 5** | | | | **9** | |

*2 bands for source library isolate; not accepted.

**Overall:** **93** **27** **50**

**Table 18. Ribotype analysis (VT/NH database) success for *E. coli* isolates with >2 bands from VT watersheds.**

| Date | 8/22/00 | | | 8/23/00 | | | 8/29/00 | | | OVERALL STUDY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | # of isolates | | | # of isolates | | | # of isolates | | | # of isolates | |
| Site | R-typed | ≥80% similarity | Source un-ID'd | R-typed | ≥80% similarity | Source un-ID'd | R-typed | ≥80% similarity | Source un-ID'd | R-typed | ≥80% similarity | Source un-ID'd |
| **Colchester area watershed** | | | | | | | | | | | | |
| C2 | 1 | 1 | 0 | 4 | 4 | 0 | 2 | 0 | 2 | 7 | 5 | 2 |
| C4 | | | | 4 | 4 | 0 | 3 | 1 | 2 | 7 | 5 | 2 |
| C5 | 2 | 1 | 1 | 5 | 5 | 0 | 1 | 1 | 0 | 8 | 7 | 1 |
| C7 | | | | 4 | 2 | 2 | 6 | 3 | 3 | 10 | 5 | 5 |
| C8 | 4 | 2 | 2 | 2 | 0 | 2 | | | | 6 | 2 | 4 |
| C9 | 1 | 0 | 1 | | | | | | | 1 | 0 | 1 |
| C12 | | | | 1 | 0 | 1 | 3 | 1 | 2 | 4 | 1 | 3 |
| C13 | 5 | 2 | 3 | 3 | 3 | 0 | 4 | 0 | 4 | 12 | 5 | 7 |
| | | | | | | | | | Total: | 55 | 30 | 25 |
| **Winooski area watershed** | | | | | | | | | | | | |
| W1 | 2 | 2 | 0 | 2 | 0 | 2 | | | | 4 | 2 | 2 |
| W2 | 2 | 1 | 1 | 1 | 1 | 0 | 5 | 1 | 4 | 8 | 3 | 5 |
| W6 | 5 | 4 | 1 | 3 | 2 | 1 | 2 | 1 | 1 | 10 | 7 | 3 |
| W8 | 4 | 3 | 1 | 2 | 1 | 1 | 4 | 1 | 3 | 10 | 5 | 5 |
| W11 | 4 | 3 | 1 | | | | 2 | 0 | 2 | 6 | 3 | 3 |
| | | | | | | | | | Total: | 38 | 20 | 18 |
| | | | | | | | | | **OVERALL TOTAI** | **93** | **50** | **43** |

**Table 19. Source species for *E. coli* (>2 bands in profile) isolated from 2 Vermont watersheds based on a combined VT/NH database.** (Dice Analysis Results: ≥80% Matching Similarity)

| Site | Total isolates | % Source species | | | | | | | | | | | | | | | % Species ID'd |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Un-ID'd | cat | chicken | cow | coyote | deer | dog | septage | gull | mallard | geese | coon | horse | fox | muskrat | |
| **Colchester watershed** | | | | | | | | | | | | | | | | | |
| C2 | 7 | 29 | | | 29 | | | | | 29 | | | 14 | | | | 71 |
| C4 | 7 | 29 | 14 | | | | | | 14 | 14 | | | 29 | | | | 71 |
| C5 | 8 | 13 | 13 | | | 13 | 13 | | 25 | | 13 | | 13 | | | | 88 |
| C7 | 10 | 50 | 10 | | | | 40 | | | | | | | | | | 50 |
| C8 | 6 | 67 | | | | | 17 | | | | | | 17 | | | | 33 |
| C9 | 1 | 100 | | | | | | | | | | | | | | | 0 |
| C12 | 4 | 75 | | | | | | | | | | 25 | | | | | 25 |
| C13 | 12 | 58 | | 17 | | | | | 17 | | | | | | | 8 | 42 |
| Totals | 55 | 44 | 6 | 4 | 4 | 2 | 11 | 0 | 9 | 6 | 2 | 2 | 9 | 0 | 0 | 2 | 56 |
| **Winooski watershed** | | | | | | | | | | | | | | | | | |
| W1 | 4 | 50 | | | | | | | 50 | | | | | | | | 50 |
| W2 | 8 | 63 | | | 13 | | | | | | | 13 | 13 | | | | 38 |
| W6 | 10 | 30 | 10 | | 10 | | 10 | | 20 | | | | 10 | 10 | | | 70 |
| W8 | 10 | 50 | 20 | | | | 10 | | 10 | | | | 10 | | | | 50 |
| W11 | 6 | 50 | | | | | 17 | 17 | | | | | | | 17 | | 50 |
| Totals | 38 | 47 | 8 | 0 | 5 | 0 | 8 | 3 | 13 | 0 | 0 | 3 | 8 | 3 | 3 | 0 | 53 |
| | | | | | | | | | | | | | | | | | |
| **Overall Totals** | **93** | **46** | **7** | **1** | **4** | **1** | **10** | **1** | **11** | **3** | **1** | **2** | **9** | **1** | **1** | **1** | **54** |
| # sites present: | | 13 | 5 | 1 | 3 | 1 | 6 | 1 | 6 | 2 | 1 | 2 | 7 | 1 | 1 | 1 | 12 |

## Table 20. Isolate occurrence from each source species in the 2 watersheds.

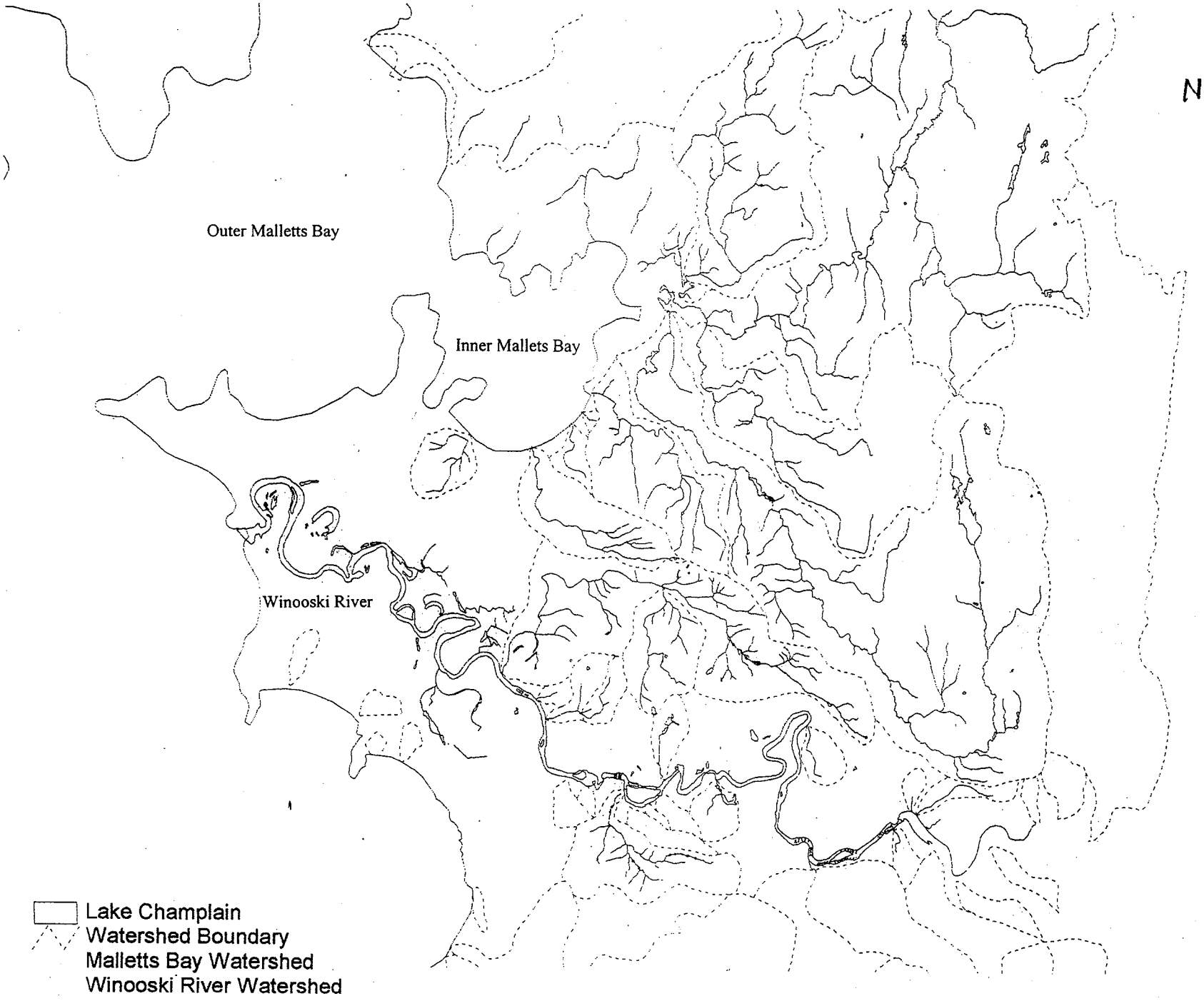
Profiles all had >2 bands, matches had ≥80% similarity.

| Source species | # of isolates | | | # of isolates | | |
|---|---|---|---|---|---|---|
| | Colchester | Winooski | Total | Colchester | Winooski | Total |
| NH +/or VT database species | **VT database** | | | **VT/NH database** | | |
| cat | 4 | 3 | 7 | 3 | 3 | **6** |
| cow | 3 | 2 | 5 | 2 | 2 | **4** |
| dog | | | | 0 | 1 | **1** |
| horse | | | | 0 | 1 | **1** |
| mallard | 1 | 0 | 1 | 1 | 0 | **1** |
| pigeon | | | | 0 | 0 | **0** |
| raccoon | 3 | 2 | 5 | 5 | 3 | **8** |
| seagull | 5 | 1 | 6 | 3 | 0 | **3** |
| septage-human | 1 | 2 | 3 | 5 | 5 | **10** |
| Total | 17 | 10 | 27 | 19 | 15 | **34** |
| Additional NH database species | | | | | | |
| chicken | | | | 2 | 0 | **2** |
| coyote | | | | 1 | 0 | **1** |
| deer | | | | 6 | 3 | **9** |
| fox | | | | 0 | 1 | **1** |
| goose | | | | 1 | 1 | **2** |
| muskrat | | | | 1 | 0 | **1** |
| Total | | | | 11 | 5 | **16** |
| **TOTAL** | **17** | **10** | **27** | **30** | **20** | **50** |

**Table 21. Isolate occurrence from different types of source species.**

Profiles all had >2 bands, matches had ≥80% similarity.

| Type of source species | Colchester | | Winooski | | **Overall study** | |
|---|---|---|---|---|---|---|
| | # of isolates | % in watershed | # of isolates | % in watershed | # of isolates | % in study area |
| **Septage**-humans | 6 | 11% | 5 | 13% | **11** | **12%** |
| **Pets** (dog, cat) | 3 | 5% | 4 | 11% | **7** | **8%** |
| **Birds** (mallard,pigeon,seagull,geese) | 5 | 9% | 1 | 3% | **6** | **6%** |
| **Livestock** (horse, cow)/chickens | 4 | 7% | 3 | 8% | **7** | **8%** |
| **Wildlife** (raccoon,coyote,deer,fox,muskrat) | 12 | 22% | 7 | 18% | **19** | **20%** |
| **Unknowns** | 25 | 45% | 18 | 47% | **43** | **46%** |
| Total | **55** | | **38** | | **93** | |

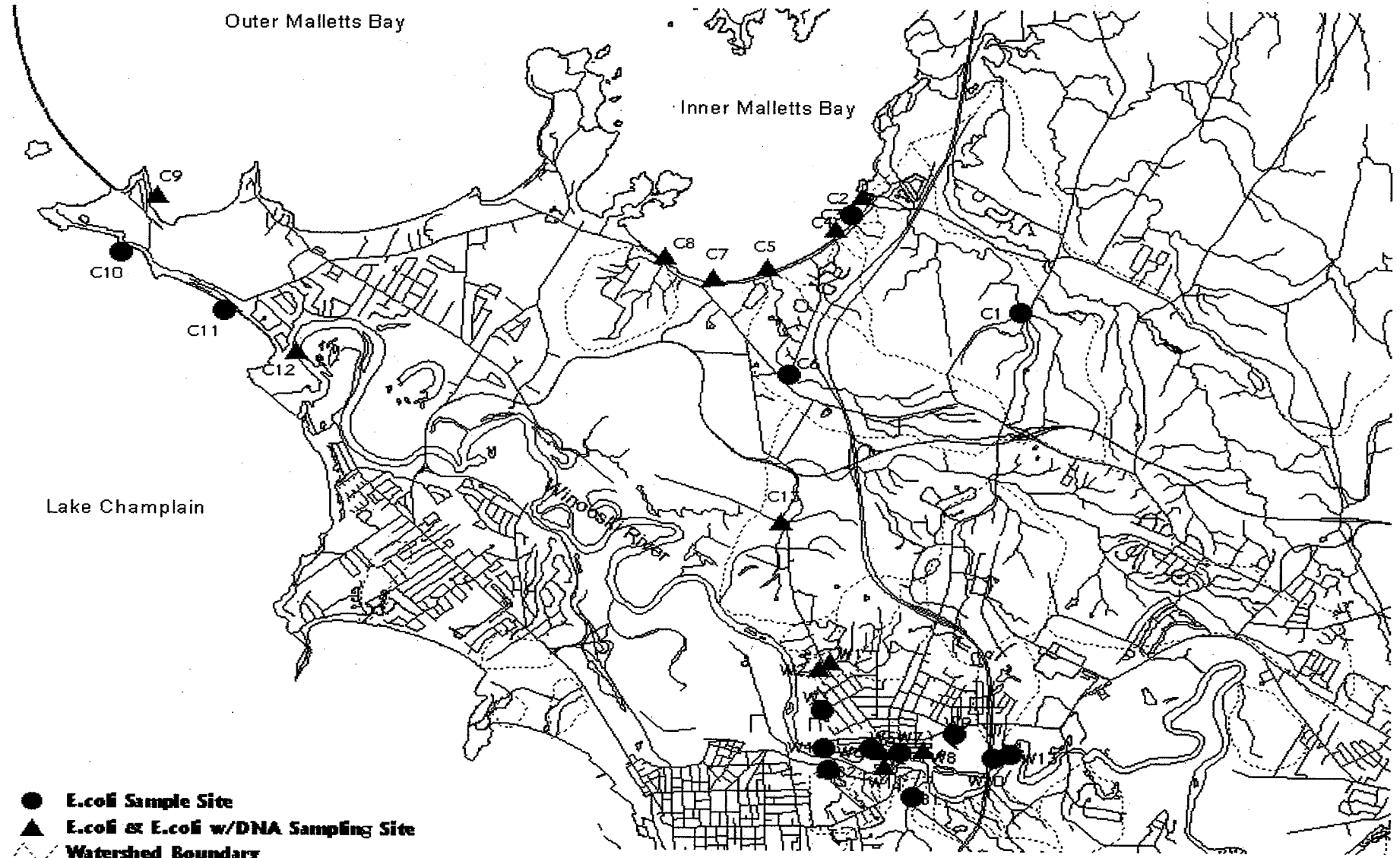



Note: original shows watersheds in color

Figure 1. Location Map

Malletts Bay and Lower Winooski Watersheds

Figure 2


Outer Malletts Bay
Inner Malletts Bay
Lake Champlain
Winooski River
C1
C2
C4
C5
C7
C8
C9
C10
C11
C12
E.coli Sample Site
E.coli & E.coli w/DNA Sampling Site
Watershed Boundary
Surface Waters
Roads
0 0.5 1 Miles
Malletts Bay and Lower Winooski River
Bacteria Reduction Study